PATENT ABSTRACTS OF JAPAN

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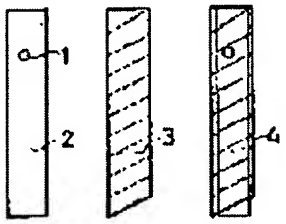
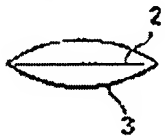
(54) IMMUNOCHROMATOGRAPHY AND DEVICE FOR IT

(57)Abstract:

PURPOSE: To eliminate the need for separating cell constituents and to analyze constituents in blood using whole blood by a development support where the development rate of the cell constituent differs from that of a liquid constituent.

CONSTITUTION: After a specimen is developed into a development support 2 by an antigen or an antibody where gold colloid is connected by the immunochromatography, generation of an antigen antibody reaction is judged according to the presence or absence of the integration of the gold colloid. Then, the development support 2, where the development rate of the cell constituent differs from that of the liquid constituent, is used. For immobilizing the antigen or antibody for the development support 2, an antigen or antibody liquid solution may be spotted and adsorbed. A spot position 1 should be the one where a complex of a substance to be measured and a gold colloid reagent passes and the cell constituent does not reach. Also, since the gold colloid reagent which is developed to the development support 2 rises while being uniformly dissipated on the development support 2, the sport position 1 should be at a low position where more gold colloids pass for improved sensitivity. The section of a sheath 3





is in lemon shape, thus protecting the inside development support and preventing a capillary phenomenon at the flat surface part of the development support.

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CLAIMS

[Claim(s)]
[Claim 1] The immunity chromatography method characterized by using the expansion base material from which the expansion rate of a cell component and an acidity-or-alkalinity component is different in the immunity chromatography method which judges generation of an antigen-antibody reaction by the existence of accumulation of gold colloid after using the antigen or antibody which combined gold colloid and making an expansion base material develop a specimen.
[Claim 2] The immunity chromatography test method given in the 1st term of a claim characterized by fixing the antibody to the matter which should be measured in the suitable location of an expansion base material using the gold colloid which combined the antibody to the matter which should be measured.
[Claim 3] The immunity chromatography test method given in the 1st term of a claim characterized by fixing the antigen to the antibody which should be measured in the suitable location of an expansion base material using the gold colloid which combined the antigen to the antibody which should be measured.
[Claim 4] The immunity chromatography test method given in the 1st term of a claim a given specimen is a whole blood sample.
[Claim 5] The immunity chromatography test method given in the 1st term of a claim with which the expansion base material from which the expansion rate of a cell component and an acidity-or-alkalinity component is different infiltrates silica gel into a glass fiber, and is prepared.
[Claim 6] The immunity chromatography test method given in the 1st term of a claim the given expansion base material from which the expansion rate of a cell component and an acidity-or-alkalinity component is different is the glass fiber filter paper which performed organic binding processing.
[Claim 7] The trial implement which fixes the antibody or antigen which causes the matter which should be measured in the suitable location of the expansion base material from which the expansion rate of a cell component and an acidity-or-alkalinity component is different, and an antigen-antibody reaction, and comes to contain the expansion base material concerned in the sheath the cross section of whose is a lemon mold thru/or a spindle mold mostly.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] About the trial implement used for an immunity chromatography test method and this, even if this invention does not separate a cell component into a detail from a whole blood sample further, it relates to the trial implement used for the immunity chromatography test method and this which can judge existence of the quality of the specified substance macroscopically.
[0002]
[Description of the Prior Art] Conventionally, the analysis method of the component using antigen-antibody reactions, such as enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), radioimmunoassay (RIA), a condensation method, and the immunity chromatography examining method, in the living body is known well. Among these, the approach which used the gold colloid (henceforth a "gold colloid reagent") combined with the antigen or the antibody as an immunity chromatography examining method is learned.
[0003] However, since this approach was what judges existence of the quality of the specified substance when gold colloid reagents gathered for the fixed part, application in a whole blood sample was made very difficult. That is, although it consisted of cell components, such as an erythrocyte, a leucocyte, and a platelet, and an acidity-or-alkalinity component, when a whole blood sample was used, a whole blood sample has the fault that existence of gold colloid cannot judge with the naked eye, and was made indispensable [separating an acidity-or-alkalinity component according to centrifugal separation etc.].
[0004] Although such separation can be carried out in the hospital where the facility was ready that there is no problem in any way, it is an environment without such a facility and the solution was called for that it must examine immediately in many cases.
[0005] In recent years, the spot of the whole blood sample is carried out between the lower limit of the expansion base material which has the trial band which made the antigen or the antibody fix as an immunity chromatography test method using a whole blood sample, and a trial band, the buffer solution which subsequently contains a gold colloid reagent from a lower limit is developed, and the method of judging accumulation of the gold colloid in a trial band is reported (Provisional Publication No. No. 25553 [63 to]).
[0006]
[Problem(s) to be Solved by the Invention] However, there is a possibility that a result may change with the amounts of spots of a whole blood sample, by this approach, moreover, since there were few those amounts of spots, remarkable skill is required and it was hard to call it a simple approach, and it was more simple and offer of the high approach of practicality was called for.
[0007]
[Means for Solving the Problem] As a result of inquiring wholeheartedly that this invention person should improve an immunity chromatography test method in view of the above-mentioned actual condition, a specific expansion base material has a big difference in the expansion rate of the cell component in blood, and an acidity-or-alkalinity component, Although the acidity-or-alkalinity

component of the expansion base material concerned is developed, a cell component and by establishing a judgment part in the location which is not developed observation is possible in accumulation of gold colloid with the naked eye, without being covered with a cell component -- becoming -- a whole blood sample -- even when it remained as it is, a header and this invention were completed for the ability of an immunity chromatography test method to be performed.

[0008] That is, after the first purpose of this invention uses the antigen or antibody which combined gold colloid and makes an expansion base material develop a specimen, it offers the immunity chromatography method characterized by using the expansion base material from which the expansion rate of a cell component and an acidity-or-alkalinity component is different in the immunity chromatography method which judges generation of an antigen-antibody reaction by the existence of accumulation of gold colloid. Moreover, the second purpose of this invention offers the trial implement which can be used for operation of the above-mentioned approach.

[0009] As an example of the expansion base material from which the expansion rate of a cell component and an acidity-or-alkalinity component used in this invention is different The expansion base material prepared through the glass fiber filter paper which performed the expansion base material which silica gel was infiltrated into the glass fiber generally known as instant thin-layer chromatography, and was prepared, and organic binding processing is mentioned. As these examples, what is marketed as instant thin-layer chromatography (ITLC; germane Saiensu-Sha make) or glass fiber filter paper GS-25 (Advantec Toyo Kaisha, Ltd. make) is mentioned.

[0010] Moreover, the gold colloid reagent used by this invention can use what is already adopted with the immunity chromatography test method. Preparation of this gold colloid reagent should just make an antibody or an antigen stick to the gold colloid marketed.

[0011] What is necessary is to carry out the spot of an antigen or the antibody solution, and just to make it stick to immobilization of the antigen or antibody to an expansion base material nonspecific.

[0012] If the location (henceforth a "spot location") which carried out the spot of an antigen or the antibody solution in this invention is a location which a cell component develops, since distinction of condensation of the gold-colloid reagent by the naked eye becomes difficult and it is meaningless, it needs to consider as the location at which the complex of the matter which should measure a spot location, and a gold-colloid reagent is passed, and a cell component does not arrive. Moreover, since the gold colloid reagent developed by the expansion base material goes up while the all do not go up to the upper limit of an expansion base material but are distributed by homogeneity on an expansion base material, it is made into as low the location (the near maximum upper limit where a cell component goes up) where more gold colloid passes through a spot location as possible, and it is [direction] highly sensitive and it is desirable.

[0013] As an element relevant to this spotting, the amount of samples (the amount of developing solutions), the classes (a hematocrit value, specific difference, etc.) of sample, the amount of gold colloid reagent solutions, the width of face of an expansion base material and thickness, the measuring time, etc. should be mentioned, and it should be set experimentally preferably.

[0014] In addition, in this invention approach, in order to lessen the antigen in a test portion, or nonspecific adsorption of an antibody, it is desirable to block the whole expansion base material which carried out the spot of an antigen or the antibody solution from for example, cow serum albumin, skimmilk powder, casein, etc.

[0015] Since it is easy to damage, as for the expansion base material used by this invention approach, it is desirable to use a trial implement as shown in drawing 1 in the operation.

[0016] The condition (this invention trial implement) that C contained the expansion base material for the sheath from which A protects an expansion base material among this drawing, and B protects it in the sheath is shown. Moreover, among drawing 1 , in one, 2 shows an expansion base material and 3 shows a sheath for a spot location, respectively. Although the sheath in this Fig. put in the slanting cut and has received expansion (absorption) of a specimen, not only a configuration such but a clearance may be formed, or it may prepare one piece thru/or two or more holes, and may improve expansion of a specimen.

[0017] Drawing 2 is a drawing in which the cross section of this invention trial implement is shown. Since the cross section of a sheath 3 has a lemon form mostly, it becomes possible [preventing the capillarity in the flat-surface section of an expansion base material which is not meant] while it protects the expansion base material included in inside effectively.
[0018] Drawing 3 is a drawing in which the busy condition of the trial implement of this invention is shown. Among drawing, five are a test tube, take the mixed specimen 6 of the whole blood sample of the specified quantity, and a gold colloid reagent in it, put this invention trial implement 4 into this, and develop a specimen from a lower limit.
[0019] Drawing 4 is the drawing in which the immunity chromatography method performed using the above-mentioned this invention trial implement was shown typically.
[0020] A shows the case where the antibody in a specimen is measured, among this drawing. In the case of this drawing, you acquire first the antigen 9 to the antibody 8 (******-ed) which exists in a specimen and which should be measured, and a part of this makes it combine with gold colloid, the spot of other one section is carried out to the expansion base material 2, and it is fixed to it. Subsequently, if the antigen 9 (gold colloid reagent) combined with gold colloid is added into a specimen and it is a test liquid, ****** 8-ed and a gold colloid reagent will react within this system, and gold colloid-antibody complex will be formed. Furthermore, since the aforementioned gold colloid-antibody complex will be combined with the antigen 9 with which the antibody 8 in complex was fixed further when it arrives at a spot location although a test liquid develops the inside of an expansion base material if this test liquid is attached to the lower limit of this invention trial implement, expansion is stopped here. And when the antibody 8 more than a constant rate exists in a specimen, gold colloid is accumulated in a spot location, also with the naked eye, it can distinguish now, and existence of the antibody 8 in a specimen is shown.
[0021] Moreover, the case where the antigen in a specimen is measured is shown by the inside B of drawing. In the case of this drawing, if the antibody 11 to the antigen 10 (tested antigen) which exists in a specimen is acquired first and is hereafter made to be the same as that of the above, the tested antigen in a specimen can be measured.
[0022] According to this invention approach, it is possible to detect and analyze various kinds of components which exist in blood, for example, hormone, an enzyme, an immunoglobulin (antibody to various antigens), vitamins, a lipopolysaccharide, protein and a nucleic acid, amino acid, polypeptides, alkaloid, the steroids, amino glucosides, a complement factor and a blood coagulation factor, physic metabolite other than the above, a metabolic intermediate and its derivative, those receptors, or a cementing material out of whole blood.
[0023]
[Effect of the Invention] In performing the immunity chromatography method, since selection use of that from which the expansion rate of a cell component and an acidity-or-alkalinity component is different is carried out as an expansion base material, this invention does not need to separate a cell component. Therefore, since the component in blood can be easily analyzed using whole blood under the case where emergency is required, and the situation that there is no sufficient facility, it is very useful to a diagnosis of various kinds of diseases.
[0024]
[Example] Next, although an example is given and this invention is explained in more detail, this invention is not restrained at all by these examples.
[0025] Fruit ** Example Preparation of 1 mouse immunoglobulin joint gold colloid: 11micro of 0.2M potassium carbonate solutions 1 was added in 600micro (product made from ZAIMEDDO) of gold colloid solutions 1 with a particle size of 15nm, and it was referred to as pH9.0. The object which prepared [ml] the mouse immunoglobulin solution (2.5mg [ml] /, the product made from ZAIMEDDO, 0.02M phosphate buffer solution, pH6.4, and 0.02% sodium azide are included) in 0.1mg /with distilled water was made 611micro of this pH preparation gold colloid solution 1 60microl addition (the amount of 2 double of an initial complement).
[0026] 600micro [of 10mM phosphate buffer solutions] (pH6.4, 1% cow serum albumin, and 0.05% sodium azide are included) 1 was added for this mixture as a stabilizing agent after shaking churning.

The at-long-intervals alignment was carried out by 14500 revolutions per minute with the supercentrifuge (KUBOTA 6800) for 60 minutes, and the supernatant was removed. Settlings were re-surfaced to 100micro [of 10mM phosphate buffer solutions] (pH6.4, 1% cow serum albumin, and 0.05% sodium azide are included) l, and mouse immunoglobulin joint gold colloid was obtained.
[0027] Fruit ** Example Preparation of the 2 this invention trial implement: The 110mmx5mm cutoff line was written on instant thin-layer chromatography (200mmx50mm: germane Saiensu-Sha make), and coincidence production of the part for ten kits was carried out. From the end, the pipeter was used for the 6cm place and 3micro (2.5mg [ml] /, the product made from ZAIMEDDO, 0.02M phosphate buffer solution, pH6.4, and 0.02% sodium azide are included) of mouse immunoglobulins was dropped at it l times. Before the dropped mouse immunoglobulin dried, this was dipped for about 30 minutes into the 0.02M phosphate buffer solution (pH6.4, 1% cow serum albumin, and 4% saccharose are included).
[0028] After making it dry in an oven (about 40 degrees C) after 3 times washing in distilled water for about 2 hours, instant thin-layer chromatography was separated to 110mmx5mm. It ****(ed) to vaginate [from which the tube made from polyethylene of the shape of a cylinder with a diameter / of 4mm / and a die length of 130mm is set by coincidence at the size of instant thin-layer chromatography, and the cross section becomes a lemon mold thru/or a spindle mold mostly]. In addition, slitting is put into the part in contact with a whole blood sample, and the sheath made the part the lower limit. The sense for which the coating point of an antibody comes to a 6cm place from the bottom was equipped with the base material for expansion into the sheath, and this invention trial implement was prepared.
[0029] Fruit ** Example The mouse [rabbit anti-] antibody measurement trial using the whole-blood sample containing three cell components: The detection trial of a rabbit anti-mouse antibody was performed by the approach shown below using this invention trial implement prepared in the mouse immunoglobulin joint gold colloid prepared in the example 1, and the example 2.
[0030] (Measuring method)
The rabbit anti-mouse antibody solution (1mg [ml] /, the Cappel make, #0611-0082) by which affinity purification was carried out was diluted 60 times, 250 times, and 1000 times using the whole blood (heparin processing) extracted from the rabbit (New Zealand white kind) auricular artery, and a total of three kinds of specimens were prepared. Moreover, what does not add a rabbit anti-mouse antibody was made into the contrast sample.
[0031] Four tubes made from polystyrene (phi:14mm, H:70mm) were prepared, and a specimen and 200micro of contrast samples l were added to each. Then, 20micro [of mouse immunoglobulin joint gold colloid] l prepared in the example 1 was added to this, and it mixed with it lightly. this invention trial implement prepared in the example 2 was stood in the tube, and room temperature neglect was carried out. Condensation extent (it appears as purple) of gold colloid was observed after about 30-minute neglect. This result is shown in Table 1.
[0032] (Measurement result)

表 1

試　料	判定結果
対照試料	陰性
60倍希釈試料	陽性
250倍希釈試料	陽性
1000倍希釈試料	陽性

[0033] In the above-mentioned trial, 5cm and an acidity-or-alkalinity component were able to go up from the lower limit to 11cm, and the cell component could distinguish the condensation location and cell component of gold colloid, and has judged them clearly. The spot location of a mouse immunoglobulin is a 6cm place from a lower limit, and judged what checked condensation (it appears as

purple) of gold colloid into the part to be a positivity.

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] The drawing in which the configuration of this invention trial implement is shown. The condition (this invention trial implement) that C contained the expansion base material for the sheath from which A protects an expansion base material among drawing, and B protects it in the sheath is shown.
[Drawing 2] The drawing in which the cross section of this invention trial implement is shown.
[Drawing 3] The drawing in which the busy condition of the trial implement of this invention is shown.
[Drawing 4] The drawing in which the device of the immunity chromatography method of this invention was shown typically.
[Description of Notations]
1 Spot Location 7 Gold Colloid
2 Expansion Base Material 8 Antibody
3 Sheath 9 Eight Antigens
4 This Invention Trial Implement 10 Antigen
5 Test Tube 11 Antibody to 10
6 Specimen
with -- Top

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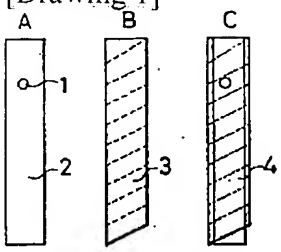
DRAWINGS

[Drawing 1]



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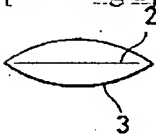
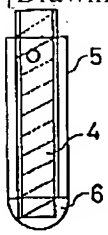


[Drawing 3]



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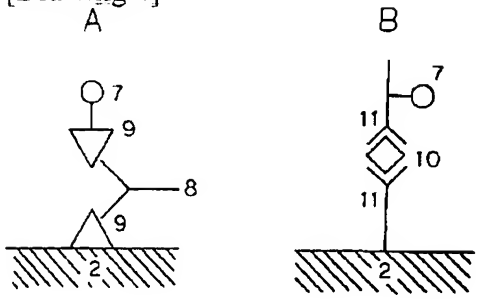

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